An RNA-Based Information Superhighway in Plants

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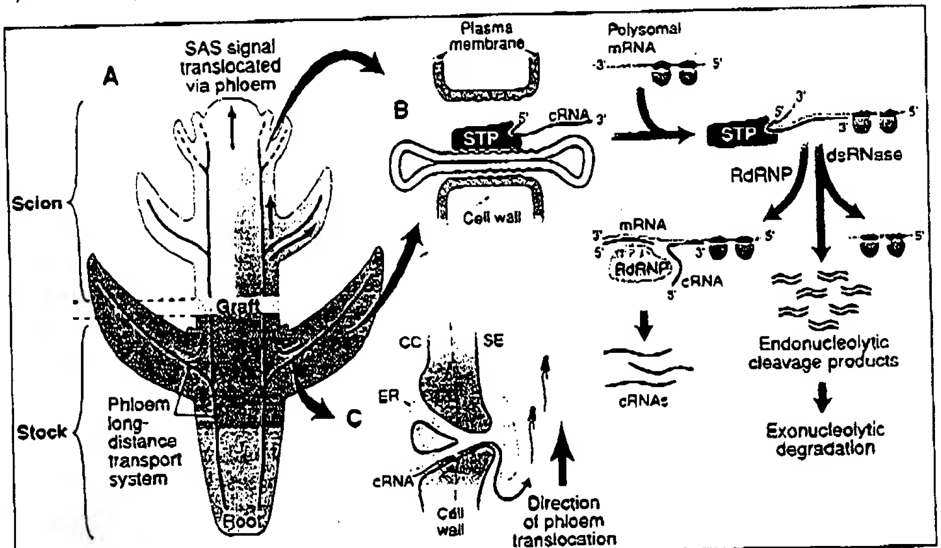
Transgenic plants that overproduce useful products project an appealing image: Grains with increased protein content, fruits and vegetables with enhanced nutritional value...flowers with deeper colors. But in trying to create such plants by overexpressing the plant's own proteins, geneticists have often inadvertently caused the opposite result. Instead of producing large quantities of new proteins, high-expressing transgenes introduced into the plant can actually inhibit the expression of the plant's own genes by triggering sequence-specific destruction of similar transcripts. Thus, the transgene ends up silencing both its own expression and that of similar endogenous genes when the concentration of transgene transcript (mRNA) becomes too high in the cytoplasm (*1*, *2*). This unintended "cosuppression" can nonetheless be harnessed by genetic engineers—to eliminate unwanted gene expression, for example—and is used by the plant itself to inhibit protein synthesis by invading viruses.

Cosuppression can affect the entire plant, but more often it silences genes in ordered patterns that follow features of plant morphology, believed to reflect underlying prepatterns of transgene transcription (*3*). Some patterns, however, suggest that cosuppression per se might not be cell-autonomous, that is, it can be transmitted between cells, perhaps throughout the entire plant (*4*). This hypothesis was confirmed recently by Vaucheret and co-workers who grafted a normal, nonsuppressed scion (upper vegetative tissues) onto a cosuppressed stock (lower vegetative tissues and the root system) and observed that cosuppression is then induced in the scion (*5*). This transmission of cosuppression through a graft union is gene-specific and requires that a transcriptionally active, nonsuppressed transgene be present in the scion (see the figure, this page). The "signal" that transmits expression is extremely mobile and can be transmitted through as much as 30 cm of a nontransgenic interstock segment to cause cosuppression in a transgenic, nonsuppressed scion. The Vaucheret group calls this phenomenon "systemic acquired silencing" (SAS), by analogy with the well-known phenomenon of systemic acquired resistance in plants, a mechanism that offers the plant broad resistance to pathogen attack (*6*).

Systemic spread of the cosuppression state has also been demonstrated in other ways. When one leaf of a plant expressing the green fluorescent protein (GFP) from jellyfish is infiltrated with an *Agrobacterium tumefaciens* strain carrying a GFP gene within its transfer DNA (T-DNA) (*7*), this T-DNA integrates into the nuclear genome of cells in the exposed leaf. Although the bacterium and the T-DNA are restricted to the infiltrated leaf, GFP expression is silenced throughout the plant.

Together these results point to the existence of a gene-specific, mobile signal molecule that transmits the cosuppression state through the plant's long-distance transport system, the phloem, and from the phloem into the surrounding tissues. The phloem is composed of enucleate sieve tube cells, which serve as a conduit for nutrient delivery throughout the plant. In addition to sugars and amino acids, the phloem contains proteins that move from leaves to the developing shoots and flowers. The precise identity of the molecule that carries the signal for cosuppression is unknown. A likely candidate is an RNA molecule derived from the suppressed gene or its transcripts and transported from cell-to-cell through plasmodesmata (*5*), the unique intercellular, cytoplasmic channels that interconnect plant cells. This hypothesis is consistent with the recent finding that plasmodesmata engage in the selective cell-to-cell trafficking of proteins and their transcripts (*8*), thereby regulating plant growth and development and orchestrating physiological function (*9*, *10*).

Plant viruses have evolved to exploit this endogenous cell-to-cell transport system to potentiate the spread of viral infection both locally and systemically within host plants (*11*). RNA viruses carrying sequences homologous to a transgene can be both targets and triggers of cosuppression. This suggests that a primary function of cosuppression is to destroy viral RNA–associated transcripts whenever they are expressed at "excessive" levels (*1*). Systemic spread of the cosuppression state, via the plant's macromolecular trafficking system, would seem to be a further evolutionary response to such viruses, allowing the plant to identify, track, and destroy viral RNA molecules in a sequence-specific manner. Consistent with this view is evidence that plants have a sequence-specific mechanism for recovery from infection by vi-



Information transfer through the plant. **(A)** Long-distance (phloem) transmission of the cosuppression state. **(B)** Model for plasmodesmal trafficking and propagation of an RNA surveillance signal within tissues expressing the transgene. STP, surveillance translocation protein (facilitates cRNA cell-to-cell and long-distance trafficking); RdRNP, RNA-dependent RNA polymerase; dsRNase, double-stranded ribonuclease. **(C)** cRNA-STP complex entering from the companion cell (CC) to the sieve element (SE) of the phloem (ER, endoplasmic reticulum).

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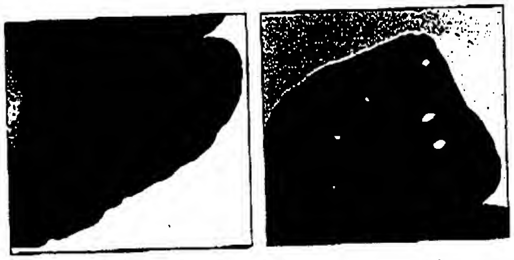
rises that allows them to resist infection (12). As with cosuppression, RNA is the target of this recovery mechanism.

Many plant viruses produce mosaic patterns on leaves during systemic infection. These mosaics are composed of dark green "islands" of healthy cells in a sea of yellow-green or white infected cells (see the figure, below) (13). The dark green islands contain little or no infectious virus, no detectable viral proteins or double-stranded RNAs, and are immune to superinfection (inoculation with large amounts of the same virus). These islands arise by cell-to-cell spread of the superimmune state during leaf development, which produces nonclonal patches of healthy cells. The immunity of dark green islands, like cosuppression, is based on sequence-specific targeting of viral RNA (14). Thus, dark green islands may be due to cell-to-cell transmission of RNA molecules that trigger a cosuppression-like state in the cells they enter, creating supracellular domains of resistance to the invading virus.



Islands of calm. (Left) Dark green islands in a sea of light green, RNA virus-infected, cells in a tobacco leaf. **(Right)** Nonclonal spots in a petunia petal caused by cosuppression of the chalcone synthase gene, required for production of purple anthocyanin pigment.

Similar mosaic phenotypes are produced by transgenes that control flower color. Mosaics occur at high frequency for transgenes that can excise from the genome late in petal development to form an extrachromosomal episome that replicates, potentially producing a burst of new transcripts (15, 16). The mosaic pattern is composed of many, randomly placed pairs of cosuppression spots in the upper and lower petal epidermis, as if a sphere of cosuppression has radiated from the point at which the transcript threshold was exceeded and cosuppression was triggered. As the wave of cosuppression moves outward and passes through cells in which pigment production has begun, a gradient of pigment marks the position of the wave. Thus, the size of the spot reflects both the time in development when cosuppression was triggered and the rapidity with which the cosuppression signal can move through plasmodesmata.

What RNA molecules could transmit the cosuppression state between cells? Candidates include bits of transcripts produced during RNA degradation (17), malformed transcripts (18), or copy RNA (cRNA) molecules produced from sense transcripts by endogenous RNA-dependent RNA polymerases (1). Although any of these RNAs could transmit cosuppression cell to cell and in the phloem, cRNA offers the greatest potential for amplifying and transmitting cosuppression state and resonates pleasingly with the notion that cosuppression may have originated in the evolution of resistance to viruses.

Conceivably, a ribonucleoprotein (RNP) complex made of cRNA molecules and plant proteins could be responsible for transmitting the signal into surrounding cells through plasmodesmata (see the figure, previous page). The RNP complex might also include a ribonuclease or an RNA-dependent RNA polymerase, postulated to be the molecular basis of transcript turnover in cosuppression (1).

This RNP complex could mediate the turnover of all homologous transcripts in the cell into which it has moved, producing more cRNA molecules from additional sense RNA templates, if present. These could move into neighboring cells, thereby resulting in progressive cell-to-cell travel of RNP surveillance complexes. This trafficking would proceed through cells in which the transcript concentration is too low to trigger suppression, as long as sufficient template is present to amplify and transmit the SAS signal. Assuming that the endogenous components of the surveillance system are expressed throughout the plant, the trafficking process would be self-perpetuating in any tissues in which sufficient template mRNA is present. Even in the absence of any template molecules, the specialized conducting cells of the phloem could rapidly translocate an RNA or RNP complex over long distances. For recovery from viral infection, cRNA molecules might act as templates to produce more cRNA molecules in systemic leaves where viral RNA has yet to arrive. The efficiency of this synthesis together with the stability of the cRNA or RNP will determine how long protection persists.

Plants that permit unrestricted movement of macromolecular complexes could be susceptible to a viral pathogen moving through and damaging whole plant. Hence, strong selection would exist for establishing limits on transmission of such complexes, just as there are endogenous restrictions preventing viral genomes from moving into plant meristems (11). Once plants could regulate the trafficking of these information molecules to direct RNA turnover, they could adapt this mechanism for the supracellular regulation of endogenous gene expression. Indeed, the sucrose transporter protein mRNA in the phloem of potato and tobacco (19) may function both as a physiological signal and as a template for protein synthesis.

Fundamental questions remain: Is the transported signal simply an RNA molecule, or is it an RNP complex composed of an endogenous movement protein and perhaps a double-stranded RNase and an RNA-dependent RNA polymerase? Characterization of the SAS signal could be a breakthrough for understanding pathogen-plant interactions. Most significant, in our view, would be the demonstration that SAS is merely "the tip of the iceberg," that it reflects the existence of an underlying supracellular surveillance system fundamental to plant development and physiology that forms a basis for sophisticated processing and transmission of large amounts of information within organs and throughout the plant.

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